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(54) Title: COMPOSITION OF ANTIBODIES AGAINST HEPATITIS B SURFACE ANTIGEN

(57) Abstract

A composition comprising at least two different human monoclonal antibodies against hepatitis B surface antigen. Combination of a human monoclonal antibody which binds to a linear epitope, e.g. MAb 4-7B, and a human monoclonal antibody which binds to a conformational epitope, e.g. MAb 9H9. The composition is useful for treatment of chronic hepatitis B patients, e.g. those having received liver transplantation in order to prevent re-infection.

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Composition of antibodies against hepatitis B surface antigen

Field of the invention

The invention is in the field of therapeutic compositions for use with patients having chronic hepatitis B and relates in particular to pharmaceutical compositions comprising antibodies against the hepatitis B surface antigen (HBsAg), which compositions are useful e.g. to prevent re-infection of liver cells in chronic hepatitis B patients after liver transplantation.

Background of the invention

Hepatitis B is an infectious disease, progressing in distinct phases. In the first phase after infection, the Hepatitis B virus replicates actively. This phase is characterized by liver damage, probably caused by immune reactivity against virus-infected liver cells. Once the immune reactivity develops sufficiently, active virus replication subsides. During the second phase (virus latency), fragments of the virus can still be detected in the blood, but inflammation of the liver gradually declines. In the last (recovery)-phase, hepatitis B virus is no longer detectable in the blood; at the same time, serum antibodies directed against surface antigens of the virus can be demonstrated.

In patients with chronic hepatitis B, transition from phase 1 to phase 2, or from phase 2 to phase 3 is delayed, and the hepatitis virus itself or virus fragments can be detected in the blood. In phase 1 in particular, there is a persistent inflammation of the liver with a risk of liver cirrhosis, which may lead to liver malfunction or liver carcinoma. Moreover, the blood of such patients is highly infectious.

During the last decade, liver transplantation has become available as a possible therapeutic modality in advanced liver disease caused by hepatitis B virus, providing a chance for long-term survival of more than 50%. Because in cases of chronic hepatitis B a transplanted liver is nearly always re-infected, with a recurrence of the original disease, the survival of hepatitis B patients after liver transplantation is much worse than the overall average in other patient groups treated similarly.

Antibodies against the hepatitis B surface antigen have been proven very effective in the prevention of hepatitis B virus infection in individuals without earlier exposure to the virus. Such antibodies may result from active vaccination or can be passively administered. However, immunization with hepatitis B vaccine or administration of anti HBs antibodies has no clear effect in patients with chronic hepatitis B.

It has been published that in HBsAg-positive patients who underwent liver transplantation in view of severe liver damage caused by the infection, intravenous administration of excessive amounts of anti HBsAg antibodies can neutralize the virus, and can thus prevent re-infection (references 1 and 2).

Indeed, prevention of re-infection of liver cells can only be achieved if a large excess of anti HBsAg antibodies can be administered: the antibodies first have to neutralize the surface antigen of the hepatitis B virus, after which a level of at least 100 International Units (IU) per liter must be maintained in the serum. Therefore, for the initial neutralization about 50,000 to 100,000 IU are required and to maintain a sufficient level during a year, at least another 100,000 IU are required. These data are based upon clinical studies in patients with chronic hepatitis B who, after liver transplantation, were preventively treated with classical, polyclonal human anti-HBs antisera (refs. 1 and 2).

Polyclonal human anti HBs antibody preparations are prepared from pooled plasma of immunized donors. These preparations are available in relatively small amounts and costly, resulting in the

overall cost of treatment of from NLG 50,000.- to NLG 100,000.- per patient. Beside this, it would be virtually impossible to obtain sufficient amounts of antibody in order to maintain in the patients adequate antibody levels to prevent re-infection of a transplanted liver.

Furthermore, it has recently been demonstrated that sometimes potential donors with anti-HBs antibodies are also infected with hepatitis C virus and therefore cannot be used for preparation of the plasma pool, resulting in a further reduction of the available anti-HBs polyclonal preparation. Until now, such re-infection nearly always occurs in such patients.

During the last decade, administration of monoclonal antibodies has evolved as a therapeutic modality. However, it is the general experience that administration of murine monoclonal antibodies in humans often leads to problems: in immunocompetent recipients, an antibody response against mouse immunoglobulin molecules occurs. This may result in several untoward effects: in any case, repeated administration of murine antibody will be impossible.

Therefore, much effort is invested in the construction of human monoclonal antibodies. Since they are homologous proteins, they will generally not elicit an antibody response in humans. The existing technology for the construction of HuMAb-producing cell lines has often turned out to be unsuccessful, however.

Further, a drawback of monoclonal antibodies is that they are nearly always directed against a single antigenic epitope, in contrast to "classical" polyclonal antisera, which contain a mixture of antibodies, recognizing many different epitopes. As a result, polyclonal antisera are better equipped to deal with the possible modifications of the virus as compared to the use of a single monoclonal antibody. It may be possible to circumvent this drawback by using a mixture of monoclonal antibodies, provided however that suitable antibodies are available which recognize different epitopes.

European patent application EP-A 0038642 discloses monoclonal antibodies against hepatitis B surface antigen which are secreted by the hybridoma cell lines RF-HBs-1, RF-HBs-2, and RF-HBs-4. The monoclonal antibody secreted by the cell line RF-HBs-1 is said to recognize an epitope which is common to the ad and ay subtypes of HBsAg and is different from the epitopes recognised by the RF-HBs-2 and RF-HBs-4 antibodies. The antibodies are claimed to have therapeutic, prophylactic, and diagnostic uses in respect of hepatitis B virus infections and said to be useful for purifying hepatitis B surface antigen. They may be used in combination with eachother. It is not claimed nor suggested that a mixture of these monoclonal antibodies could neutralize hepatitis B virus almost as efficiently as a polyclonal antiserum.

Said known antibodies are mouse monoclonal antibodies produced by hybridomas resulting from the fusion of spleen cells from suitably immunized Balb/c mice and a mouse myeloma cell line. Therapeutic use of such mouse monoclonal antibodies is expected to result in an inhibitory immune response after parental application. Furthermore, said known antibodies recognize only one of the subtypes of hepatitis B. This is particularly undesirable with an eye on neutralization of escape mutants, which are less likely to occur in the case of two antibodies reactive with the same subtype.

Brief description of the drawing

Figure I shows the results of AUSRIA inhibition experiments carried out for polyclonal anti HBs antiserum (HBIg);
Figure II shows the results of AUSRIA inhibition experiments carried out for human monoclonal antibody MAb 4-7B;
Figure III shows the results of AUSRIA inhibition experiments carried out for human monoclonal antibody MAb 9H9;
Figure IV shows the results of AUSRIA inhibition experiments carried out for a 1:1 mixture of human monoclonal antibodies MAb 9H9 and MAb 4-7B (on the basis of weight);
Figure V shows the results of AUSRIA inhibition experiments carried out for a 10:1 mixture of human monoclonal antibodies MAb 9H9 and MAb 4-7B (on the basis of weight);

Figure VI shows the results of AUSRIA inhibition experiments carried out for a 1:10 mixture of human monoclonal antibodies MAb 9H9 and MAb 4-7B (on the basis of weight).

Summary of the invention

Human monoclonal antibodies against HBs (HuMAb-anti HBs) have been developed by immortalization of antibody-producing cells from HBs-immune individuals. Human monoclonal antibodies having specificity for different epitopes have been obtained. The antibodies can be obtained in high amounts and are relatively inexpensive. It has been shown that a well-chosen combination of at least two of said human monoclonal antibodies recognizing different epitopes will effectively inhibit the binding of polyclonal human anti-HBsAg antibodies. In view thereof, such a combination promises to be a suitable alternative for the use of a polyclonal antiserum in the treatment of chronic Hepatitis B patients.

Detailed description of the invention

This invention provides a pharmaceutical composition comprising antibodies against hepatitis B surface antigen and a pharmaceutically acceptable carrier or diluent, wherein said antibodies comprise at least two different human monoclonal antibodies.

In a preferred embodiment of the invention, said human monoclonal antibodies include a first human monoclonal antibody and a second human monoclonal antibody, wherein binding of said first human monoclonal antibody to hepatitis B surface antigen causes less than 50% inhibition of binding of said second human monoclonal antibody to said hepatitis B surface antigen, and binding of said second human monoclonal antibody to hepatitis B surface antigen causes less than 50% inhibition of binding of said first human monoclonal antibody to said hepatitis B surface antigen.

According to the invention, it is especially preferred that one of said human monoclonal antibodies binds to a linear epitope of

hepatitis B surface antigen and another human monoclonal antibody binds to a conformational epitope of hepatitis B surface antigen. Most preferably, the human monoclonal antibody binding to a linear epitope of hepatitis B surface antigen is MAb 4-7B (deposited under the Budapest Treaty with the European Collection of Animal Cell Cultures on 10 December 1992, deposit number ECACC 92121016) and the human monoclonal antibody binding to a conformational epitope of hepatitis B surface antigen is MAb 9H9 (deposited under the Budapest Treaty with the European Collection of Animal Cell Cultures on 10 December 1992, deposit number ECACC 92121015).

This invention provides the above pharmaceutical composition for use in the treatment of chronic hepatitis B patients.
The invention also provides a human monoclonal antibody against hepatitis B surface antigen for use in the treatment of chronic hepatitis B patients, in particular MAb 4-7B (ECACC 92121016) or MAb 9H9 (ECACC 92121015).

In addition, the invention provides the use of a human monoclonal antibody against hepatitis B surface antigen for preparing a pharmaceutical composition for the treatment of chronic hepatitis B patients, more in particular use of human monoclonal antibodies MAb 4-7B (ECACC 92121016) and MAb 9H9 (ECACC 92121015) for that purpose.

The present invention also provides a process of producing a human monoclonal antibody against hepatitis B surface antigen, said process comprising culturing cells which produce monoclonal antibody MAb 4-7B (ECACC 92121016), or cells which produce monoclonal antibody MAb 9H9 (ECACC 92121015), and optionally recovering the monoclonal antibody produced. Preferably, said cells are cultured in vitro in a nutrient medium therefor and the antibody produced is recovered from the culture supernatant.

With respect to the selection of the cells which produce said monoclonal antibodies, various alternatives exist. Preferably said cells are from the deposited cell line MAb 4-7B (ECACC 92121016), or from the deposited cell line MAb 9H9 (ECACC 92121015), or are

hybrid cells derived from any one of said deposited cell lines by fusion with a murine myeloma cell line, or are recombinant cells transformed with nucleic acid encoding any one of monoclonal antibodies MAb 4-7B (ECACC 92121016) and MAb 9H9 (ECACC 92121015) such as to allow expression thereof.

Details concerning the preparation of human monoclonal antibodies and formulation of therapeutic antibody compositions for treating chronic hepatitis B patients will not be given herein since they belong to the normal knowledge and routine of persons skilled in the art and are extensively documented in the literature.

The invention will be illustrated in the following examples which are not intended to limit the invention but merely serve the aim to illustrate it.

Example 1: Preparation of human monoclonal antibodies against HBs

Several HuMAb-anti-HBs were prepared as follows: Peripheral blood lymphocytes were isolated from volunteer plasmapheresis donors who had high titres of serum antibodies against HBs. The B lymphocytes were isolated and infected with Epstein-Barr virus, resulting in the transformation and immortalization of antibody-producing cells. By repeated sub-culture of the immortalized cells, monoclonal cell lines (i.e. genetically identical) were isolated. Such cell lines continuously secrete HuMAb-anti-HBs. Large amounts of these antibodies are readily obtained from the supernatant of these cells when cultured in-vitro.

Example 2: examination of two HuMAb-anti-HBs

The properties of two of the HuMAb-anti-HBs prepared in accordance with Example 1 are described herein in some detail. The two HuMAb-anti-HBs are designated herein as CLB-Hu-HBsAg-1 (MAb 4-7B) and CLB-Hu-HBsAg-4 (MAb 9H9).

2.1 Specificity of MAb 4-7B and MAb 9H9

The specificities of the antibodies were tested in a radio-immunoprecipitation assay: the antibodies were coupled to a solid phase, which was then incubated with radioactive, ^{125}I-labelled HBs proteins (obtained from Abbott Laboratories, Chicago, ILL, USA). The bound, labelled proteins were then analyzed by means of SDS polyacrylamide gel electroforesis (PAGE), after which the separated proteins were detected by means of auto-radiography.

It was found that both antibodies react with all known major HBs proteins (i.e. major (p24/gp27), middle (gp33/gp36) and large (gp39/gp42)). In an immuno-blotting technique with denaturated HBs, only MAb 4-7B showed reactivity, while MAb 9H9 did not bind. This finding indicates that MAb 4-7B reacts with a denaturation-resistant determinant (i.e. linear epitope), whereas MAb 9H9 detects a denaturation-sensitive determinant (i.e. conformation epitope).

In order to confirm the finding that two different epitopes are recognized by the two antibodies, cross-competition radioimmuno-assays were performed. To this end, both monoclonal antibodies were radioactively labelled with ^{125}I. Next, HBs-coated particles were pre-incubated with an excess of unlabelled MAb 4-7B, followed by labelled MAb 9H9 and vice versa. These results are as follows:

antibody during pre-incubation (in excess)	antibody for labelling	% binding of label (mean of two experiments)
MAb 4-7B	MAb 4-7B	1.5
MAb 4-7B	MAb 9H9	63
MAb 9H9	MAb 9H9	10
MAb 9H9	MAb 4-7B	79

Percentage binding refers to the binding of the labeled monoclonal antibody after pre-incubation with an excess of unlabeled antibody, and expressed relative to the percentage in the absence of unlabeled antibody (the latter value being taken as 100%).

Conclusions:

a) unlabeled MAb 4-7B and MAb 9H9 are able to block the binding of their labeled counterparts;

b) unlabeled MAb 4-7B hardly blocks the binding of labeled MAb 9H9, and vice versa.

These findings show that MAb 4-7B and MAb 9H9 are indeed directed against different epitopes, which is consistent with the data mentioned earlier (i.e. MAb 4-7B recognizes a linear epitope and MAb 9H9 a conformation-dependent epitope).

2.2 relevancy of the use of a mixture of MAb 9H9 and MAb 4-7B for therapy

An AUSRIA inhibition experiment was performed to demonstrate that a mixture of MAb 4-7B and MAb 9H9 has superior properties as compared to the two separate antibodies.

In this experiment, HBsAg was incubated with antibody MAb 4-7B, antibody MAb 9H9 or with mixtures containing known concentrations of both antibodies. For comparison, a polyclonal anti-HBs antiserum was tested in parallel.

Residual HBs-immunoreactivity was tested in the AUSRIA assay (test kits obtained from Abbott Laboratories). The principle of the AUSRIA is as follows: beads coated with polyclonal (guinea pig) anti-HBsAg antibodies are incubated with the plasma from a hepatitis B patient, which contains HBs, overnight at ambient temperature. After washing, known quantities of MAb 4-7B, MAb 9H9 or a mixture of both antibodies were added and incubated for 1 hour at 45°C. Then, without washing, the beads were incubated with ^{125}I-labelled polyclonal (human) anti-HBsAg antibodies for 3 hours at 45°C. Finally the binding of the labeled antibodies was measured and the capacity of the HuMAbs to inhibit the binding of the ^{125}I-labeled conjugate was calculated. The results of these experiments are shown in Tables I-VI and Figures I-VI.

These results clearly demonstrate that both MAb 9H9 and MAb 4-7B separately (Table and Figure II and III) could only partially

block the binding of the labeled polyclonal antibody. However, all mixtures of MAb 4-7B and MAb 9H9 (Table and Figure IV to VI) tested, showed complete inhibition as compared to the unlabeled polyclonal anti HBs (Table and Figure I) antiserum.

Additional experiments were performed to study whether the antibodies are able to react with Hepatitis B viral particles in a similar way as they react with isolated HBsAg. For this purpose, whole viral particles (so-called Dane particles) were used.

These Dane particles were purified from human, HBsAg-positive serum by gradient centrifugation. The reaction of the antibodies to these Dane particles was tested by incubation in solution. After incubation, AUSRIA beads were added to the solution. Furthermore, HBV-DNA was extracted from the remaining solution and analyzed semi-quantitatively by Dot-Spot hybridization. After hybridization with a radioactive probe specific for HBV, the exposure was examined visually.

The experiment with the Dane particles showed that both monoclonal antibodies 4-7B and 9H9 were able to neutralize a Dane particle preparation to a similar extent as HBsAg. For example, a 1/30000 diluted 9H9 antibody was able to yield 73% and 75% neutralization of HBsAg and Dane particles, respectively; a 1/3200 diluted MAb 4-7B preparation resulted in 64% and 74% neutralization, respectively. Complete neutralization was obtained when a mixture of monoclonal antibodies 9H9 and 4-7B was used. It was shown that this 100% neutralization corresponded with a complete removal of HBV-DNA from the Dane particle preparation.

Hence, it can be concluded that a mixture of both 4-7B and 9H9 antibodies is able to fully neutralize Dane particles as well as HBsAg. Conclusively, a mixture of MAb 4-7B and MAb 9H9 provides a suitable preparation as an alternative for the treatment of chronic Hepatitis B patients circumventing the drawbacks of the "classical" treatment with a polyclonal preparation.

The present invention has been described with particular reference to specific cell lines producing the two monoclonal antibodies 4-7B and 9H9. However, alternative cell lines producing antibodies with the same or similar binding specificities can be generated, e.g. to improve antibody production methods. These alternative cell lines can be generated in several ways.

Firstly, the producing cell line 4-7B or 9H9 can be fused with another cell line (e.g. a mouse myeloma). The resulting new cell line may be selected for improved growth and/or antibody producing characteristics. After fusion and selection, the thus made new cell line will produce the very same antibody as the parent cell line 4-7B or 9H9.

Secondly, the genetic material can be extracted from either of the two cell lines 4-7B and 9H9 in order to isolate the genes encoding the antibodies. These isolated genes (or parts of the isolated genes) can be used to transform another cell line into a cell line producing proteins with similar HBsAg binding specificities as 4-7B or 9H9 antibodies.

Consequently, various modifications of the invention in addition to those shown and described herein, apparent to those skilled in the art from the preceding description, are considered to fall within the scope of the appended claims.

References

1 Lauchart W., Müller R., Pichlmayr R. Long term immunoprophylaxis of Hepatitis B virus reinfection in recipients of human liver allografts. Transplantation Proc. 1987; Vol XIX, No. 5, pp 4051-4053.

2 Müller R., Gubernatis G., et al. Liver transplantation in HBs antigen (HBsAg) carriers, prevention of hepatitis B virus (HBV) recurrence by passive immunization. Journal of Hepatology, 1991; 13: 90-96.

TABLE I

$-\log_{(2)}$dilution	% inhibition	specific activity mIE/ml
1	99.4	62,500
2	98.9	31,250
3	98.1	15,625
4	97.0	7,812
5	94.3	3,906
6	88.2	1,953
7	75.7	977
8	42.5	488
9	26.0	244
10	9.8	122
11	13.5	61
12	-12.2	31

TABLE II

$-\log_{(2)}$dilution	% inhibition	antibody concentration ng/ml	specific activity mIE/ml
1	60.0	500,000	250,000
2	59.0	250,000	125,000
3	58.6	125,000	62,500
4	55.8	62,500	31,250
5	47.1	31,250	15,625
6	57.2	15,625	7,813
7	47.0	7,813	3,906
8	39.2	3,906	1,953
9	29.0	1,953	977
10	28.2	977	488
11	29.5	488	244
12	22.2	244	122
13	-23.7	122	61
14	0.3	61	30
15	13.8	30	15
16	5.9	15	8
17	-0.8	8	4
18	-11.7	4	2

TABLE III

$-\log_{(2)}$ dilution	% inhibition	concentration ng/ml	specific activity mIE/ml
1	91.4	500,000	7500×10^3
2	90.5	250,000	3750×10^3
3	91.4	125,000	1875×10^3
4	89.8	62,500	938×10^3
5	88.7	31,250	469×10^3
6	89.5	15,625	234×10^3
7	87.4	7,813	117×10^3
8	87.4	3,906	58.5×10^3
9	84.3	1,953	29.3×10^3
10	82.3	977	14.7×10^3
11	82.3	488	7.3×10^3
12	80.5	244	3.6×10^3
13	76.1	122	1.8×10^3
14	73.6	61	0.9×10^3
15	64.3	30	0.45×10^3
16	58.2	15	0.23×10^3
17	25.1	8	0.11×10^3
18	-26.8	4	0.06×10^3

TABLE IV

$-\log_{(2)}$ dilution	% inhibition	antibody concentration ng/ml		specific activity mIE/ml	
		MAb 9H9	MAb 4-7B	MAb 9H9	MAb 4-7B
1	98.4	250,000	250,000	3750×10^3	125×10^3
2	98.4	125,000	125,000	1875×10^3	62.5×10^3
3	98.0	62,500	62,500	938×10^3	31.3×10^3
4	97.7	31,250	31,250	469×10^3	15.6×10^3
5	97.2	15,625	15,625	234×10^3	7.8×10^3
6	96.6	7,813	7,813	117×10^3	3.9×10^3
7	95.5	3,906	3,906	58.5×10^3	2.0×10^3
8	93.8	1,953	1,953	29.3×10^3	0.97×10^3
9	93.0	977	977	14.7×10^3	0.49×10^3
10	89.0	488	488	7.3×10^3	0.24×10^3
11	85.3	244	244	3.6×10^3	0.12×10^3
12	75.8	122	122	1.8×10^3	0.06×10^3
13	76.3	61	61	0.9×10^3	0.03×10^3
14	40.0	30	30	0.45×10^3	0.02×10^3
15	33.2	15	15	0.23×10^3	0.008×10^3
16	15.1	8	8	0.11×10^3	0.004×10^3
17	-8.0	4	4	0.06×10^3	0.002×10^3
18	-72.4	2	2	0.03×10^3	0.001×10^3

TABLE V

$-\log_{(2)}$ dilution	% inhibition	antibody concentration ng/ml		specific activity mIE/ml	
		MAb 9H9	MAb 4-7B	MAb 9H9	MAb 4-7B
1	97.7	227,250	22,750	3409×10^3	11,375
2	97.6	113,625	11,375	1704×10^3	5,688
3	97.4	56,813	5,688	852×10^3	2,844
4	97.2	28,406	2,844	426×10^3	1,422
5	96.4	14,203	1,422	213×10^3	711
6	95.8	7,102	711	107×10^3	356
7	95.2	3,551	355	53.3×10^3	178
8	94.2	1,775	178	26.6×10^3	89
9	91.9	888	89	13.3×10^3	44
10	88.4	444	44	6.7×10^3	22
11	82.5	222	22	3.3×10^3	11
12	78.5	111	11	1.7×10^3	5.6
13	73.1	56	5.6	0.8×10^3	2.8
14	68.4	28	2.8	0.4×10^3	1.4
15	60.5	14	1.4	0.2×10^3	0.7
16	24.2	6.9	0.7	0.1×10^3	0.4
17	2.9	3.5	0.4	0.05×10^3	0.2
18	-5.9	1.7	0.2	0.03×10^3	0.1

TABLE VI

$-\log_{(2)}$ dilution	% inhibition	antibody concentration ng/ml		specific activity mIE/ml	
		MAb 9H9	MAb 4-7B	MAb 9H9	MAb 4-7B
1	98.4	22,750	227,250	341×10^3	114×10^3
2	98.3	11,375	113,625	171×10^3	57×10^3
3	97.5	5,688	56,813	85×10^3	28×10^3
4	96.8	2,844	28,406	43×10^3	14×10^3
5	96.4	1,422	14,203	21×10^3	7.1×10^3
6	95.2	711	7,102	11×10^3	3.6×10^3
7	93.7	355	3,551	5.3×10^3	1.8×10^3
8	92.5	178	1,775	2.7×10^3	0.89×10^3
9	90.3	89	888	1.3×10^3	0.44×10^3
10	85.3	44	444	0.67×10^3	0.22×10^3
11	66.9	22	222	0.33×10^3	0.11×10^3
12	52.8	11	111	0.17×10^3	0.055×10^3
13	25.3	5.6	56	0.08×10^3	0.028×10^3
14	5.5	2.8	28	0.04×10^3	0.014×10^3
15	20.4	1.4	14	0.02×10^3	0.007×10^3
16	-0.6	0.7	6.9	0.01×10^3	0.003×10^3
17	-7.4	0.4	3.5	0.005×10^3	0.002×10^3
18	-15.5	0.2	1.7	0.003×10^3	0.001×10^3

CLAIMS

1. A pharmaceutical composition comprising antibodies against hepatitis B surface antigen and a pharmaceutically acceptable carrier or diluent, wherein said antibodies comprise at least two different human monoclonal antibodies.

2. A pharmaceutical composition according to claim 1, wherein said human monoclonal antibodies include a first human monoclonal antibody and a second human monoclonal antibody, wherein binding of said first human monoclonal antibody to hepatitis B surface antigen causes less than 50% inhibition of binding of said second human monoclonal antibody to said hepatitis B surface antigen, and binding of said second human monoclonal antibody to hepatitis B surface antigen causes less than 50% inhibition of binding of said first human monoclonal antibody to said hepatitis B surface antigen.

3. A pharmaceutical composition according to claim 1, wherein one of said human monoclonal antibodies binds to a linear epitope of hepatitis B surface antigen and another human monoclonal antibody binds to a conformational epitope of hepatitis B surface antigen.

4. A pharmaceutical composition according to claim 3, wherein said human monoclonal antibody binding to a linear epitope of hepatitis B surface antigen is MAb 4-7B (ECACC 92121016) and said human monoclonal antibody binding to a conformational epitope of hepatitis B surface antigen is MAb 9H9 (ECACC 92121015).

5. A pharmaceutical composition according to any one of the claims 1 to 4 for use in the treatment of chronic hepatitis B patients.

6. A human monoclonal antibody against hepatitis B surface antigen for use in the treatment of chronic hepatitis B patients.

7. A human monoclonal antibody according to claim 6, which is MAb 4-7B (ECACC 92121016) or MAb 9H9 (ECACC 92121015).

8. Use of a human monoclonal antibody against hepatitis B surface antigen for preparing a pharmaceutical composition for the treatment of chronic hepatitis B patients.

9. Use of human monoclonal antibodies MAb 4-7B (ECACC 92121016) and MAb 9H9 (ECACC 92121015) for preparing a pharmaceutical composition for the treatment of chronic hepatitis B patients.

10. A process of producing a human monoclonal antibody against hepatitis B surface antigen, comprising culturing cells which produce monoclonal antibody MAb 4-7B (ECACC 92121016), or cells which produce monoclonal antibody MAb 9H9 (ECACC 92121015), and optionally recovering the monoclonal antibody produced.

11. A process according to claim 10, wherein said cells are cultured in vitro in a nutrient medium therefor and the antibody produced is recovered from the culture supernatant.

12. A process according to claim 10 or claim 11, wherein said cells are from the deposited cell line MAb 4-7B (ECACC 92121016), or from the deposited cell line MAb 9H9 (ECACC 92121015), or are hybrid cells derived from any one of said deposited cell lines by fusion with a murine myeloma cell line, or are recombinant cells transformed with nucleic acid encoding any one of monoclonal antibodies MAb 4-7B (ECACC 92121016) and MAb 9H9 (ECACC 92121015) such as to allow expression thereof.

HBIg

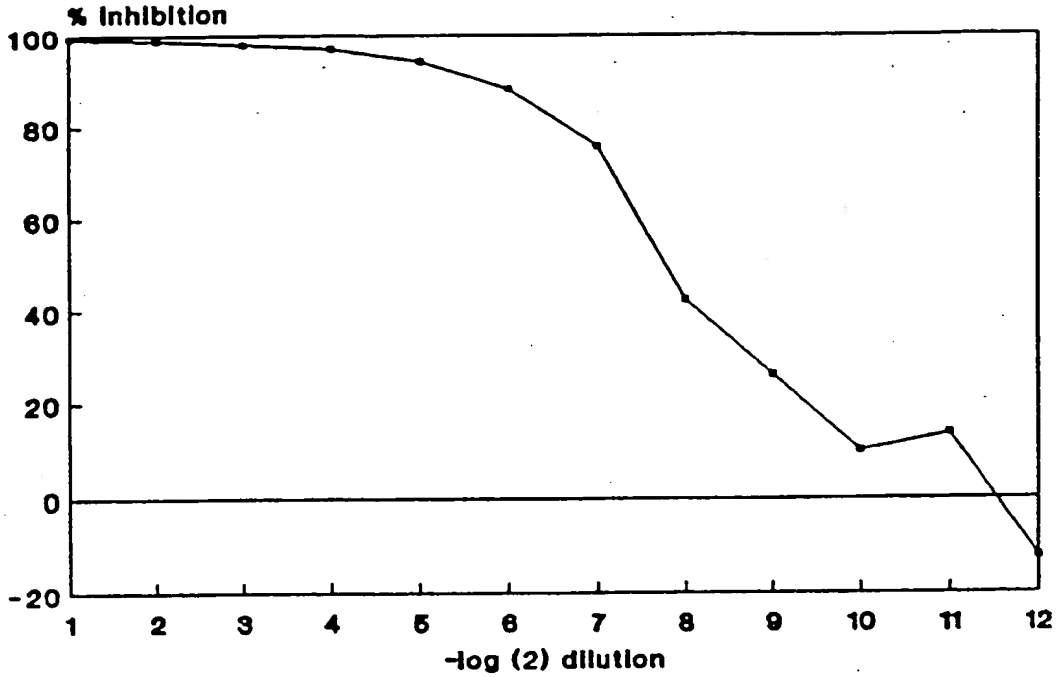

FIG.1

4-7B

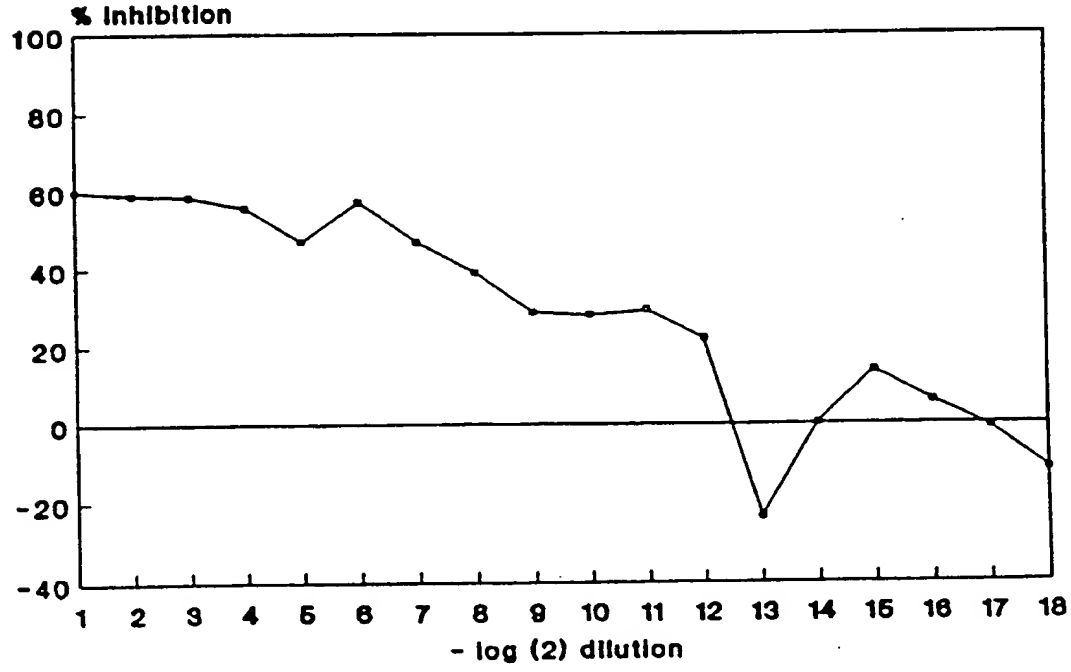

FIG.2

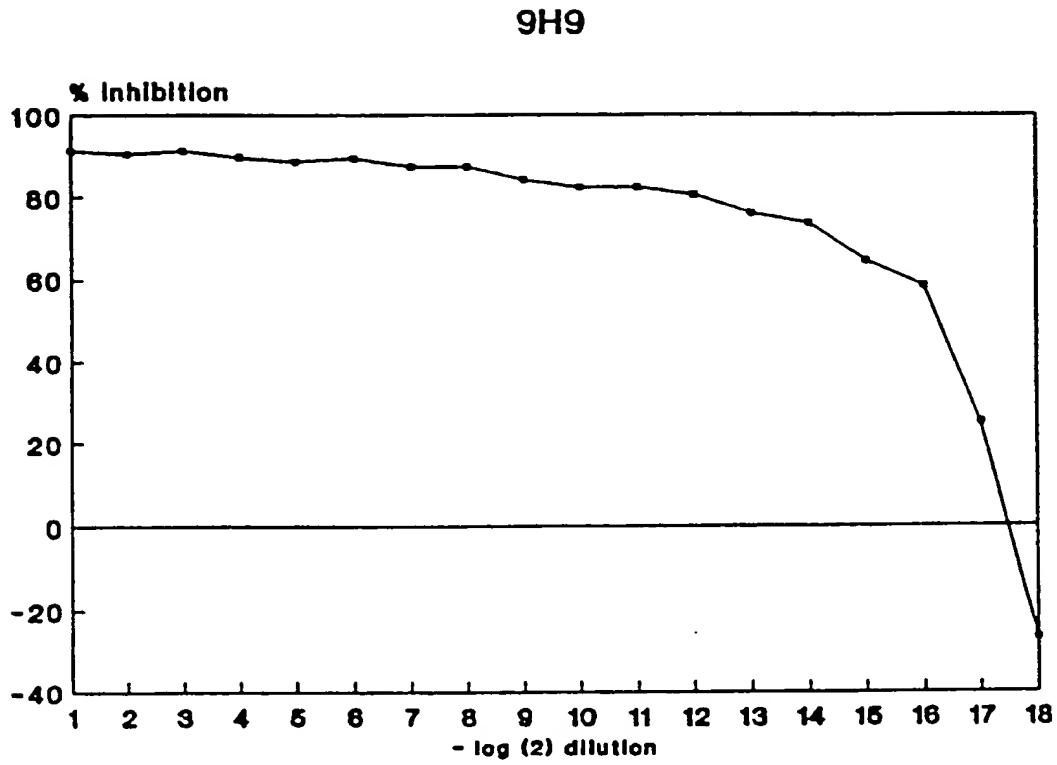
9H9
% inhibition
100
80
60
40
20
0
-20
-40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
- log (2) dilution

FIG. 3

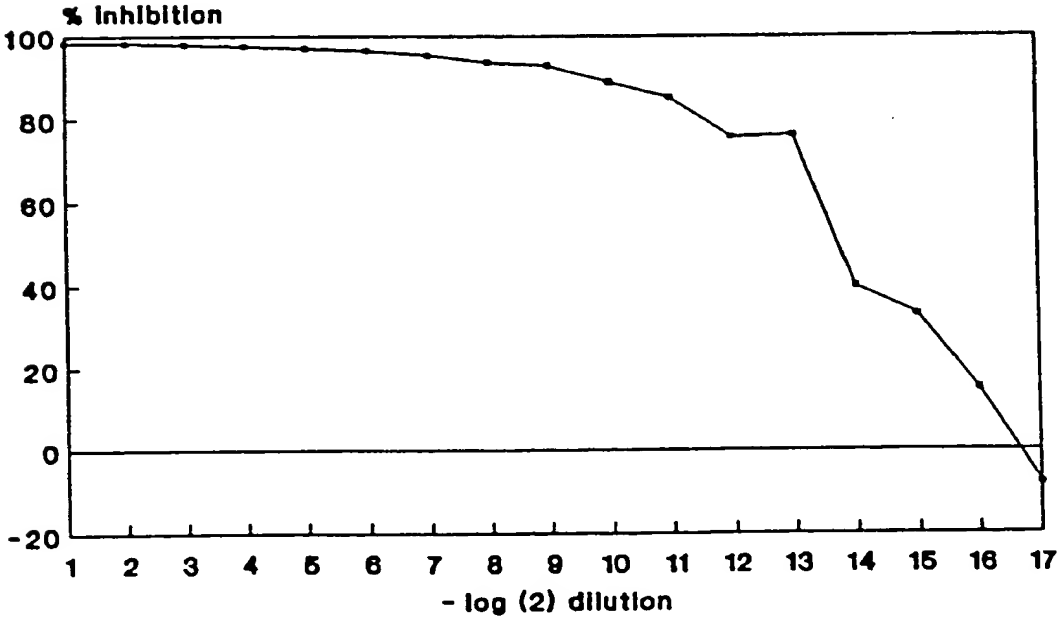
9H9/4-7B Mixture
(1:1)
% inhibition
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
- log (2) dilution

FIG. 4

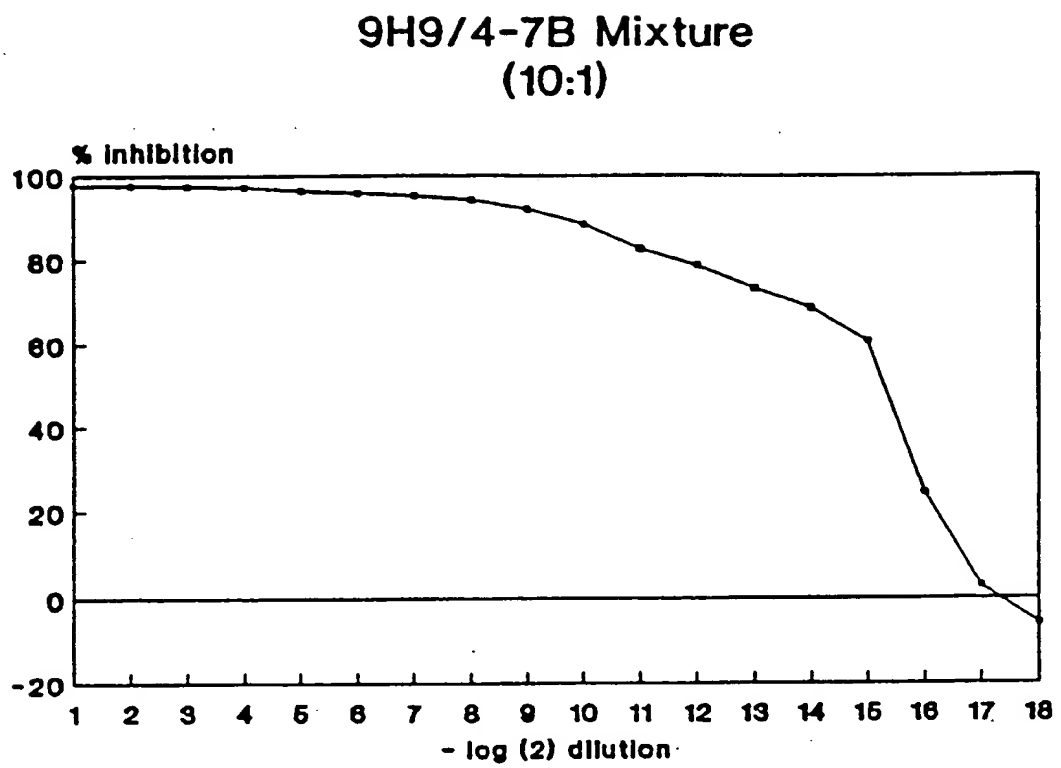
9H9/4-7B Mixture
(10:1)
% inhibition
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
- log (2) dilution

FIG.5

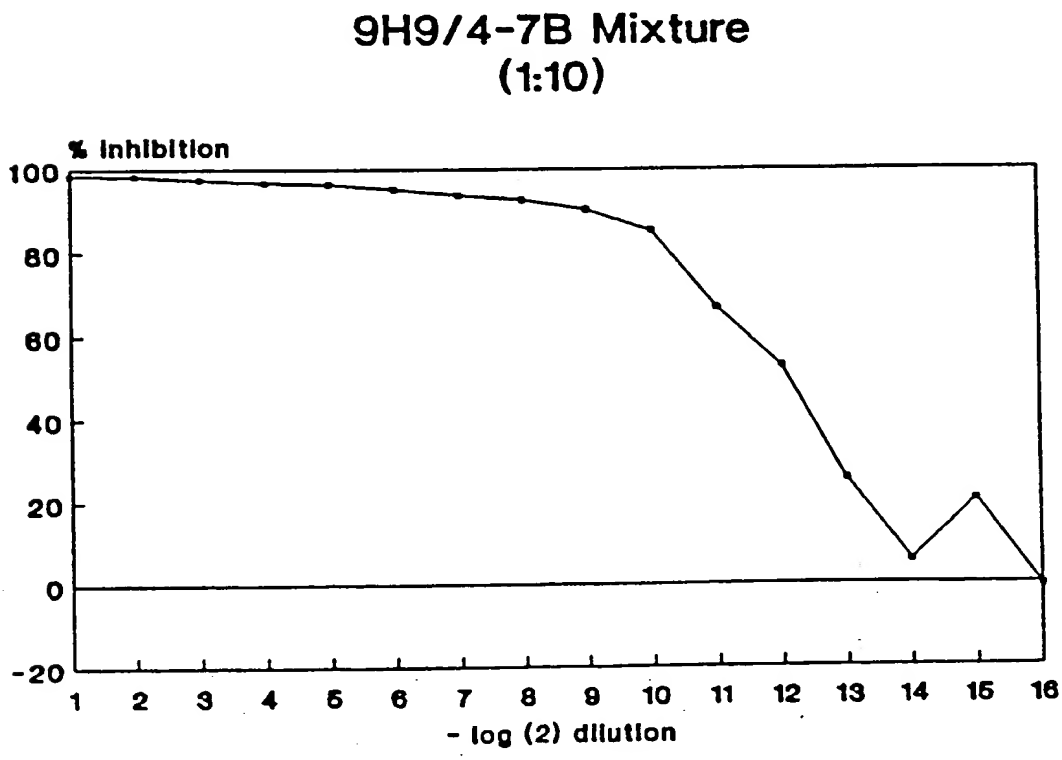
9H9/4-7B Mixture
(1:10)
% inhibition
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
- log (2) dilution

FIG.6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 94/00102

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K15/00 A61K39/42 A61K39/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	DATABASE WPI Week 9422, Derwent Publications Ltd., London, GB; AN 94-183497 & WO,A,94 11495 (SANDOZ LTD) 26 May 1994 see abstract	1,2,5,8
X	EP,A,0 038 642 (NATIONAL RESEARCH DEVELOPMENT CORPORATION) 28 October 1981 cited in the application see page 3, line 22 - page 4, line 19 see page 15, line 10 - page 16, line 4 see page 19, line 1 - page 20, line 5; example 4	1,2,5,8
A	EP,A,0 235 805 (THE ROYAL FREE HOSPITAL SCHOOL OF MEDICINE) 9 September 1987 see the whole document	1-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 September 1994	

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 99, no. 19, 7 November 1983, Columbus, Ohio, US; abstract no. 156566u, R. W. MOYLE ET AL 'A circular antibody-antigen complex is responsible for increased affinity shown by mixtures of monoclonal antibodies to human chorionic gonadotropin' see abstract & J. Immunol. 1983, 131(4), 1900-5 ---	1-12
A	CHEMICAL ABSTRACTS, vol. 106, no. 11, 16 March 1987, Columbus, Ohio, US; abstract no. 82808p, R. L: CERIANI ET AL 'Experimental immunotherapy of human breast carcinomas implanted in nude mice with a mixture of monoclonal antibodies against human milk fat globule components' see abstract & Cancer Res. 1987, 47(2), 532-40 -----	1-12

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 94/00102

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		EP-A,B 0232921	19-08-87
		JP-B- 6040833	01-06-94
		JP-A- 56158718	07-12-81
		JP-A- 2276573	13-11-90
		JP-B- 6034712	11-05-94
		US-A- 5204095	20-04-93
EP-A-0235805	09-09-87	AU-A- 6969187	10-09-87
		JP-A- 62281824	07-12-87